Supplementary Table 1. Luminescence intensity of leaves, flowers and calluses of tobacco plants expressing components of the fungal bioluminescent system. n = number of biologically independent plant samples (leaves, flowers, callus pieces) imaged in a single experiment. Detailed version of this table in Microsoft Excel format is available as Supplementary Data.

Species	Genotype	Tissue	Mean total flux, photons/min/cm2	Standard deviation	n
Nicotiana benthamiana	*nnhisps, nnh3h, nnluz, nncph,* KanR	leaf	**5.58×10^9**	1.40×10^9	19
		flower	**6.47×10^{10}**	1.60×10^{10}	5
		callus	**3.90×10^{10}**	1.60×10^{10}	24
Nicotiana tabacum	*nnhisps, nnh3h, nnluz, nncph,* KanR	leaf	**4.31×10^9**	8.44×10^8	33
		flower	**3.17×10^{10}**	4.76×10^9	5
		callus	**1.97×10^{10}**	6.21×10^9	6

Supplementary Table 2. Primers used for plant genotyping

Gene	Primer sequences	Amplicon size
nptII (KanR)	5'- GCTATGACTGGGCACAACAGACAATC -3' 5'-TCCGAGTACGTGCTCGCTCGA -3'	381 bp
nnluz	5'-CAATAGCATTCCCAATTATCCGAAGAG-3' 5'- ACAATCTTACCAGCAGGATCGTTAGTCA-3'	691 bp
nncph	5'-GTAGAGAAGGTAAGACAATTCAAGCATACGA -3' 5'-TCTTCTCGAACTGTATTTGCGAGAGTTC-3'	775bp
nnhisps	5'-TGGATGTATTTCTCGACACGGCTAGA-3' 5'-TCAGCTCTGTCGGATATGTTGAAGGA -3'	735 bp
nnh3h	5'-AGCATCAAAGGATGACTTGTTTCGAGT-3' 5'-GCTGAGTTAGAGCTCCTAAGCAAGGT-3'	465 bp

Supplementary Table 3. Concentration of caffeic acid and hispidin in leaves and flowers of *Nicotiana tabacum* plants measured by LC-MS/MS (µg/g of dry weight).

Nicotiana tabacum line	**Genotype**	**Caffeic acid in leaves at 3 am, µg/g (mean ± SD)**	**Caffeic acid in leaves at 3 pm *, µg/g (mean ± SD)**	**Caffeic acid in flowers, µg/g (mean ± SD)**	**Hispidin in flowers**, µg/g (mean ± SD)**
NT000	Wild type *Nicotiana tabacum*	2.51±0.46	2.32±0.25	0.76±0.004	<0.05
NT001	*nnhisps, nnh3h, nnluz, nncph,* KanR	2.24±0.63	1.56±0.29	0.73±0.24	0.08±0.02
NT078	*nnh3h-nnluz* coding for fusion protein, KanR	no data	1.80±0.12	no data	no data

* The content of caffeic acid was different in the wild type tobacco NT000 and transgenic line NT001 at 3 pm ($p \leq 0.05$, Student's t-test).

** In leaves of *N. tabacum*, we also found trace amounts of hispidin, both in the wild type and transgenic lines. Hispidin was only detectable if we concentrated sample 20-fold with solid-phase extraction cartridges, however, this procedure negatively affected reproducibility of measurements.

Supplementary Note 1. Engineering of caffeic acid cycle pathway in plants.

Neonothopanus nambi caffeic acid cycle produces and metabolises 3-hydroxyhispidin from caffeic acid. The reactions are catalysed by four enzymes: luciferase nnLuz, hispidin synthase nnHispS, hispidin-3-hydroxylase nnH3H and oxyluciferin recycling enzyme nnCPH (Figure 1).

Since residual amounts of hispidin were found in wild-type *N. tabacum* plants (Supplementary Table 3), we first created a plant line constitutively expressing fusion protein nnH3H-nnLuz. This strain did not emit light in a self-sustained manner but was luminescent upon injection of hispidin or luciferin.

As the 4'-phosphopantetheinyl transferase activity required for posttranslational modification of nnHispS [1] is likely present in tobacco [2,3], and caffeic acid is abundant in plants [4], we hypothesised that constitutive expression of only three enzymes may be sufficient for self-sustained bioluminescence and that further addition of the luciferin-recycling enzyme may increase the metabolic efficiency of the pathway.

Indeed, expression of three genes, *nnhisps*, *nnh3h* and *nnluz*, was sufficient to make tobacco plants autonomously bioluminescent. Additional expression of putative luciferin recycling enzyme nnCPH did not increase the brightness of plants suggesting the existence of a kinetic bottleneck at another enzymatic step in the caffeic acid cycle or a different function for the enzyme. For most experiments in this study we used the line NT001 containing two copies of the construct (Supplementary Figure 14).

Supplementary Note 2. Toxicity of caffeic acid cycle

The overall phenotype of transgenic plants was similar to the wild type plants suggesting that unlike bacterial bioluminescent system [5], high expression of caffeic acid cycle is not toxic and does not impose an obvious burden on plants (Supplementary Figure 3). More detailed analysis revealed minor differences in carotenoid content and plant height, and no difference in leaf shape and size and chlorophyll content. *N. tabacum* plants of both transgenic and wild-type lines proceeded from the vegetative state of *in vitro* culture to the flowering stage within the 8th week after the transfer to greenhouse. While we did not collect quantitative data on seed germination, no obvious difference was observed between wild type and transgenic lines. We also noticed that exposure to intense sunlight resulted in more abundant areas of necrosis in older leaves of transgenic plants in comparison to the wild-type plants.

Supplementary Note 3. Can hispidin precursors pass through cell membranes?

Hispidin biosynthesis is a multi-component multistep reaction that requires caffeic acid, malonyl-CoA and ATP.

Cell membranes are not passively permeable to coenzyme A derivatives. It is not clear whether plant cells can actively uptake malonyl-CoA from the environment.

Another component of the reaction is caffeic acid. Plant cells are able to uptake phenolic acids [6] and amino acids [7] from the environment.

Another compound, ATP, cannot passively cross the membranes. Tracer experiments confirmed influx of externally applied ATP into plant cells [8] but extracellular ATP is also known to act as a signalling molecule in plants[9].

In addition, there is a negative feedback in phenolic metabolism: caffeic acid is known as PAL inhibitor [19]. After infiltration with caffeic acid, its biosynthesis may be arrested until the excess of caffeic acid is metabolised, explaining why intensity of luminescence at the sites of injection drops slightly below the initial level before injection.

Supplementary Note 4. Activity and regulation of phenylpropanoid metabolism in plants.

Availability of phenolic acids in plants is controlled through expression of genes of central phenylpropanoid pathway coding for phenylalanine ammonia-lyase, cinnamic acid 4-hydroxylase and p-coumaric acid 3-hydroxylase [10,11], as well as by expression of enzymes that use phenolic acids as substrate (Figure 1). The phenylpropanoid metabolism is additionally shaped by tissue-specific distribution of numerous isoforms of these enzymes, compartmentalization, metabolic channeling and expression of membrane transporters [12]. Enzymes of phenylpropanoid pathway are typically associated in metabolons to increase reaction rates, localise toxic intermediates and channel metabolism towards different competing branches of biosynthesis [12]. In central phenylpropanoid metabolism, phenylalanine ammonia-lyase colocalizes with cinnamic acid 4-hydroxylase and 4-coumarate:CoA ligase on the cytosolic side of the endoplasmic reticulum membrane [12,13] limiting leakage of intermediates into the cytosol.

Steady-state concentration of luciferin in glowing plants should depend on metabolic flux through phenylpropanoid metabolism being limited by the concentration of caffeic acid available to HispS. Production of caffeic acid reflects the activity of the central phenylpropanoid metabolism [14]. Commitment to this pathway is controlled by phenylalanine ammonia-lyase. Its promoters exhibit developmental control and are active in roots, in particular, in vascular and endodermal tissues of lateral roots, leaf and petal tips, pigmented regions of petals and stamen filaments [15,16]. Similarly, expression profiles of C4H and C3H (CYP98A3) include anthers, apical part of pistils and lignifying tissues: leaf veins, stem vascular bundles and root central cylinder [17]. High expression of isoforms of 4-Coumarate:CoA Ligase (4CL) in *Arabidopsis* was also revealed in lignified tissues. 4CL4 was reported to be expressed in the epidermis, cortex, cambium, phloem, and pith. 4CL1 and 4CL3 activity was detected in flowers and roots [18]. These patterns overlap with the observed distribution of luminescence in glowing plants (Figure 2, Supplementary Figures 4, 10, 15, 16)

Supplementary Note 5. Spatial patterns of luminescence

The brightest parts of the young shoots were the terminal and axillary buds and the upper part of the stem. As plants matured, older parts of the shoot dimmed (Supplementary Video 5). During flowering the glow of flower buds surpassed luminescence from other parts of the plant. Luminescence was brighter in the petals and particularly the ovary, and apical portions of the style and stamen filaments (Figure 2c, 2e, Supplementary Figure 10). Notably, the distribution of luminescence resembled reported expression patterns for phenylalanine ammonia-lyase [15], an entry point of the phenylpropanoid pathway (Supplementary Note 4). These observations suggested that light intensity is linked to metabolic activity and might reflect the availability of caffeic acid.

Supplementary Note 6. Circadian oscillations of luminescence

nnluz gene was expressed at high levels throughout the day, with time-dependent oscillations (Supplementary Figure 17). The expression decreased during the night (21:00 to 6:00) and increased after the sunrise (05:45 on the day of collection).

Experiments with continuous night (Supplementary Figure 13c, 13d) revealed two peaks of luminescence in complete darkness, corresponding to the subjective day of plants. This indicates that the luminescence is controlled by the circadian clock in tobacco. The subjective time also affects nyctinastic leaf movements as estimated by oscillations of the top line on Supplementary Figure 13c.

The subsequent two dark 24-hour periods lack any circadian oscillation of luminescence, correlated to nyctinastic movement deprivation. These observations indicate that in tobacco plants the circadian clock is effectively switched off on the third and fourth 24-hour periods in the dark.

The circadian rhythm of both luminescence and nyctinastic leaf movements restores completely within two days of normal lightening (Supplementary Figure 13c, 13d).

Supplementary Note 7. Other temporal patterns of luminescence

In *N. tabacum*, bright bioluminescence often appeared in leaves as flickering dots or ovals with a diameter of a few millimeters. This flickering pattern was especially evident in young leaves, particularly those near brightly glowing pruning-induced axillary shoots. Imaging of the lower side (abaxial surface) of these leaves revealed luminescence dynamics suggesting intercellular transfer events possibly associated with phloem transport (Supplementary Video 8). Finally, in young leaves, we observed dynamic waves of bright luminescence spreading throughout the blades during the day (Supplementary Figure 15, Supplementary Video 9). Homogeneous distribution of signal, not associated with the leaf vascular system, suggests a transport-independent mechanism behind the increase in light emission and may correspond to previously reported spatiotemporal waves of gene expression [19].

References

1. Kotlobay, A.A. et al. *Proc. Natl. Acad. Sci. U. S. A.* **115**, 12728–12732 (2018).
2. Sierro, N. et al. *Nat. Commun.* **5**, 3833 (2014).
3. Yalpani, N., Altier, D.J., Barbour, E., Cigan, A.L. & Scelonge, C.J. *Plant Cell* **13**, 1401–1409 (2001).
4. Li, L. et al. *Sci. Rep.* **6**, 37976 (2016).
5. Krichevsky, A., Meyers, B., Vainstein, A., Maliga, P. & Citovsky, V. *PLoS One* **5**, e15461 (2010).
6. Kobayashi, A., Kim, M.J. & Kawazu, K. *Z. Naturforsch. C* **51**, 527–533 (1996).
7. Berlin, J. & Widholm, J.M. *Phytochemistry* **17**, 65–68 (1978).
8. Shang, Z., Laohavisit, A. & Davies, J.M. *Plant Signal. Behav.* **4**, 989–991 (2009).
9. De Col, V. et al. *Elife* ***6***:e26770, (2017).
10. Zhang, X. & Liu, C.-J. *Mol. Plant* **8**, 17–27 (2015).
11. Camm, E.L. & Towers, G.H.N. *Phytochemistry* **12**, 961–973 (1973).
12. Biała, W. & Jasiński, M. Frontiers in Plant Science **9**:1610, (2018).
13. Achnine, L., Blancaflor, E.B., Rasmussen, S. & Dixon, R.A. *Plant Cell* **16**, 3098–3109 (2004).
14. Vogt, T. *Mol. Plant* **3**, 2–20 (2010).
15. Kawamata, S. et al. *Plant Cell Physiol.* **38**, 792–803 (1997).
16. Sriprasertsak, P. & Raweerotwiboon, A. Kasetsart J. (Nat. Sci.) **36**, 361-369 (2002).
18. Li, Y., Kim, J.I., Pysh, L. & Chapple, C. *Plant Physiology* 00838.2015 (2015).doi:10.1104/pp.15.00838
19. Wenden, B., Toner, D.L.K., Hodge, S.K., Grima, R. & Millar, A.J. *Proc. Natl. Acad. Sci. U. S. A.* **109**, 6757–6762 (2012).